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Analysis of Clinical and Food-Borne Isolates of *Listeria monocytogenes* in the United States by Multilocus Enzyme Electrophoresis and Application of the Method to Epidemiologic Investigations

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To investigate the microbiology and epidemiology of the 1,700 sporadic cases of listeriosis that occur annually in the United States, we developed a multilocus enzyme electrophoresis (MEE) typing system for *Listeria monocytogenes*. We studied 390 isolates by MEE. Eighty-two electrophoretic types (ETs) were defined. Two distinct clusters of ETs, ET group A (ETGA) and ET group B (ETGB), separated at a genetic distance of 0.440, were identified. Strains of ETGB were associated with perinatal listeriosis ($P = 0.03$). All strains of H antigen type a were in ETGA, while all strains of H antigen type b were in ETGB. Among 328 clinical isolates from cases of listeriosis, 55 ETs of *L. monocytogenes* were defined. Thirty-four ETs were identified among 62 isolates from food products. The mean number of strains per ET (5.2) was significantly higher among clinical isolates than among food-borne isolates. Examination of isolates from outbreaks further documented the link between cases and contaminated food products. In one investigation, we found 11 different ETs, ruling out a single common source as a cause of that outbreak. By examining a large number of isolates collected over a specified time in diverse geographic locations in the United States, we have begun to establish a baseline for the study of the epidemiology of listeriosis by MEE.

Three outbreaks of listeriosis in North America and one in Switzerland, each associated with consumption of a specific food item, were recognized between 1981 and 1987 (2, 11, 15, 21). In addition, an estimated 1,700 sporadic cases of listeriosis occur annually in the United States (B. Gellin, submitted for publication). The epidemiology of these sporadic infections remains obscure (12). Microbiologic methods to distinguish between strains of *Listeria monocytogenes* have included serotyping and bacteriophage typing. Serotyping of *L. monocytogenes* is based on the analysis of somatic and flagellar antigens, combinations of which have defined 16 different serotypes (serovars) of the organism (24). However, serologic classification of the organism using polyclonal antiserum is of limited value in elucidating the epidemiology of the organism, since over 90% of invasive disease is caused by serotype 1/2a, 1/2b, or 4b. Phage typing is more discriminating in distinguishing between strains and has been used successfully in several epidemiologic investigations (3, 15, 16, 17, 20), but the method suffers in that many isolates of *L. monocytogenes* are not typeable. Therefore, an alternative method of discriminating among strains of *L. monocytogenes* is needed to supplement these methods.

Multilocus enzyme electrophoresis (MEE) has recently been used to elucidate the epidemiology of several species of bacteria (6, 7, 9, 27). Since most enzymes examined are constitutive, all strains of a species may be typed by the method. Besides providing a more discriminating technique to study the epidemiology of bacterial disease, this method provides a framework for determining the overall genetic relationships among strains of a species (26). Recently, the method has been applied to the analysis of *L. monocytogenes* (4, 19). These previous studies have concentrated on analysis of clinical isolates (4) and on isolates obtained during outbreaks of listeriosis (19). Little detailed information concerning the distribution of genetic variants of *L. monocytogenes* as determined by MEE in either the United States or Europe is contained in either of the previously published works. Contamination of foods with the bacteria has been implicated in both epidemic listeriosis and sporadic listeriosis (2, 10–12, 15, 17, 21–23). Thus, to adequately apply MEE to the epidemiology of listeriosis it is necessary to ascertain the distribution of electrophoretic types (ETs) of the organism in foods. Except for the analysis of a few strains isolated from foods during investigations of listeriosis outbreaks, these data have not been reported. Here we describe the analysis of a large collection of recent clinical and food isolates of *L. monocytogenes* in the United States by MEE, discuss how these distinct populations may relate to each other, and illustrate the application of the method to epidemiologic investigations.

MATERIALS AND METHODS

Bacterial strains. We studied 390 strains of *L. monocytogenes*. Of these, 275 strains were isolated from patients with listeriosis in the United States during 1986 and 1987. Of these clinical isolates, 222 were isolated from blood, cerebrospinal fluid, or other ordinarily sterile sites during a laboratory-based surveillance study to detect cases of listeriosis in Los Angeles County, Calif., and the states of Oklahoma, Tennessee, Washington, New Jersey, and Missouri (Gellin, submitted). The remainder of the clinical strains were received by the Special Bacteriology Reference Laboratory of the Centers for Disease Control during the same period for

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identification and serotyping. Six of these isolates were from patients who had consumed contaminated food. Isolates from these six foods were also examined.

Representative clinical isolates from recent outbreaks (7 isolates from Massachusetts [1983] [11]; 15 isolates from California [1985] [15]; 2 isolates from Switzerland [2]; 3 isolates from Nova Scotia [21], Canada; and 22 isolates from Philadelphia, Pa. [1988] [23]) were also examined. In addition, isolates cultured from nonhuman primates with listeriosis (four strains) were studied. Fifty-six isolates from commercial dairy products and seafoods were obtained from the U.S. Food and Drug Administration. All isolates were confirmed as *L. monocytogenes* by standard microbiologic procedures (5). Serotyping was done by the methods of Seeliger and Hohne (24).

MEE. Strains of *L. monocytogenes* were grown for 18 h with shaking at 37°C in 100 ml of Trypticase (BBL Microbiology Systems, Cockeysville, Md.). The cells were harvested by centrifugation at 5,000 × *g* for 10 min. The cells were suspended in 2 ml of breaking buffer (10 mM Tris, 1 mM EDTA [pH 6.8], 0.038% NADP), and the cytoplasmic contents were released by vigorous vortexing with 1 ml of glass beads (75- to 150-μm diameters; Sigma Chemical Co., St. Louis, Mo.) for 2.5 min. The extracts were electrophoresed in starch gels (11.4%), and the gels were stained for specific enzyme activities as described by Selander et al. (25). The electrophoretic mobilities of the following 16 enzymes were determined: alanine dehydrogenase (ADH), isocitrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6P), NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (GP1), NADP-dependent glutamate dehydrogenase (GLD), indophenol oxidase (IPO), phosphoglucomutase (PGM), 2-alpha-naphthyl propionyl esterases 1 and 2 (NP1 and NP2), leucyl-glycyl-glycine peptidase (LGG), fumarase (FUM), aconitase (ACO), mannose-6-phosphate isomerase (MPI), phosphoglucose isomerase (PGI), acid phosphatase (ACP), and 6-phosphogluconate dehydrogenase (PGD). IDH, NP1, NP2, and ACP were assayed on pH 6.7 Tris-citrate gels; all of the other enzymes were assayed on pH 8.2 Tris-citrate gels.

Analysis of data. Relative enzyme mobilities were established for each enzyme locus by comparison with enzyme extracts of eight control strains. These strains were sufficiently variable to provide controls for most of the different alleles or electromorphs (electrophoretic variation of an enzyme) for each of the 16 enzymes assayed. A number corresponding to the relative anodal migration was assigned to each electromorph. These numbers were assigned according to increasing distance traveled from the cathode. Each unique combination of electromorphs was designated as an ET. The reproducibility of the method was ascertained by typing a set of 12 strains several times over a period of 2 years. The method of Nei (18) was used to determine genetic diversity. Matrices of weighted distance coefficients were calculated (13), and dendrograms describing genetic distances (GDs) were generated from these data (26). Genetic diversity (h) was calculated by using the formula $h = (1 - \Sigma x_i^2)[n/(n-1)]$, where x_i is the frequency of the ith allele and n is the number of ETs (26).

RESULTS

Eighty-two ETs of *L. monocytogenes* were defined. The dendrogram position number, designation, and individual enzyme profile of each ET are shown in Table 1. In this collection of *L. monocytogenes* isolates, 15 of the 16 enzymes tested were polymorphic and one (ACO) was monomorphic within the species. The distribution of these ETs among the surveillance isolates, other clinical isolates, and isolates from food products is shown in Tables 2 and 3. Pairwise comparisons of the enzyme profile of each ET with itself and with that of every other ET were made. When the number of mismatches was plotted against the number of times that number of mismatches occurred in this population of ETs, a bimodal distribution pattern was evident (Fig. 1), suggesting the existence of two genetically distinct populations of ETs.

A matrix of pairwise distance coefficients was calculated, and from these coefficients a dendrogram (Fig. 2) was generated. The *L. monocytogenes* ETs (horizontal axis) in the dendrogram are numbered consecutively from 1 to 82 for convenience. These numbers, referred to as dendrogram position numbers (DPNs), are only for convenience of discussion within the context of this report. Within our laboratory, a set of arbitrarily assigned ET numbers is maintained. These ET designations are not changed from one set of data to another and form the basis for our in-house system. These ET designations are included in Tables 1 to 3.

Examination of the dendrogram reveals two distinct clusters of ETs, separated by a GD of 0.440. These two *L. monocytogenes* clusters will be referred to as ET group A (ETGA) and ET group B (ETGB). At a GD of 0.188, the ETs can be further divided into 17 clusters, each containing one or more ETs. At this GD, the strains in ETGA are distributed primarily among three clusters of ETs: DPNs 2 to 7 (25 strains), 8 to 29 (32 strains), and 29 to 35 (16 strains). There are two large clusters within ETGB, one consisting of DPNs 46 to 64 (77 strains) and another consisting of DPNs 65 to 75 (109 strains). Table 4 lists the genetic diversities, the mean numbers of alleles per locus, and the mean numbers of strains per ET for ETGA, ETGB, and the entire collection of ETs.

Studies with 12 strains of *L. monocytogenes* during the 2-year project resulted in no misassignment of alleles of any of the 16 enzymes assayed. We found that it was of utmost importance to include controls for alleles of the enzymes assayed in each gel. In some cases (the 3 and 4 alleles of LGG and the 5 and 6 alleles of NP1), the resolution of bands was such that extracts of some strains had to be rerun next to the appropriate controls to ensure that the assignment of alleles was consistent. For enzymes that showed no activity in some strains (null alleles), the strains were re-extracted and concentrated to ascertain whether the lack of activity was due to a poorly made extract or a real variant. In all cases, the enzyme band was still not present on the gel after re-extraction. For the relatively large number of null alleles with NP2, it is possible that the enzyme comigrated with NP1 and was, as a result, masked.

Clinical isolates. Fifty-five electrophoretic types of *L. monocytogenes* were defined among the clinical isolates. Serotyping was also done for all 275 clinical isolates of *L. monocytogenes*, thereby allowing complete analysis of genetic relatedness among serotypes (Table 5). Except for three cases in which serotype 3b strains were in ETs made up of predominantly 1/2b strains (ET4, ET17, and ET22), no ET contained isolates from more than one serotype. Serotype 1/2b strains were distributed among 12 ETs, serotype 3b strains were among 5 ETs, serotype 4b strains were among 10 ETs, and serotype 1/2a strains were in 29 ETs. Single isolates of serotypes 1/2c and 3a were each in unique ETs.

Genetic diversity, mean numbers of alleles per locus, and mean numbers of strains per ET are shown in Table 5. The

TABLE 1. ET designation, DPN, and enzyme profile of *L. monocytogenes* ETs

ET[a]	DPN[a]	Relative mobility of[b]:															
		ADH	LGG	MPI	PGD	GLUD	NP1	NP2	ACP	IDH	PGI	FUM	G6P	PGM	GP1	IPO	ACO
1	46	1	2	1	1	3	6	5	4	2	2	2	2	3	2	1	1
2	57	1	2	1	1	3	6	5	4	3	2	2	2	3	2	1	1
3	49	1	3	1	1	3	4	5	3	2	2	2	2	3	2	1	1
4	61	1	4	1	1	3	5	5	2	3	2	2	2	3	2	1	1
5	76	1	4	1	1	3	5	5	2	3	2	2	2	1	2	1	1
7	52	1	5	1	1	3	6	5	3	2	2	2	2	3	2	1	1
8	51	1	3	3	1	3	4	5	3	2	2	2	2	3	2	1	1
9	63	1	5	3	1	3	6	5	3	3	2	2	2	3	2	1	1
11	77	2	3	1	1	2	6	5	4	2	2	2	2	3	2	1	1
12	65	2	3	1	1	3	6	6	3	3	2	2	2	3	2	1	1
13	67	2	3	1	1	3	6	5	3	3	2	2	2	3	2	1	1
14	73	2	4	1	1	3	6	5	3	2	2	2	2	3	2	1	1
15	66	2	4	1	1	3	6	6	3	3	2	2	2	3	2	1	1
16	75	2	5	1	1	3	5	5	2	2	2	2	2	3	2	1	1
17	68	2	3	3	1	3	5	5	3	3	2	2	2	3	2	1	1
18	69	2	3	3	1	3	6	5	3	3	2	2	2	3	2	1	1
19	64	1	5	3	1	3	6	5	3	2	2	2	1	3	2	1	1
20	55	1	3	1	1	3	4	5	3	2	2	2	2	3	1	1	1
21	78	1	3	1	1	2	1	5	2	2	2	2	2	3	2	1	1
22	58	1	3	1	1	3	6	5	4	3	2	2	2	3	2	1	1
23	50	1	3	1	1	3	5	5	3	2	2	2	2	3	2	1	1
24	79	2	5	1	1	3	6	5	4	3	1	2	2	3	2	1	1
25	56	1	3	1	2	3	4	5	3	2	2	2	2	3	2	1	1
26	81	2	3	3	1	3	5	5	3	2	3	2	2	3	2	1	1
27	53	1	4	1	1	3	3	5	3	2	2	2	2	3	2	1	1
30	62	1	3	1	1	3	4	5	2	3	2	0	2	3	2	1	1
31	54	1	4	1	1	3	4	5	3	3	2	0	2	3	2	1	1
32	60	1	5	1	1	3	6	5	3	3	2	3	2	3	2	1	1
33	59	1	3	1	1	3	6	5	3	3	2	3	2	3	2	1	1
34	47	1	4	1	1	3	6	5	4	3	2	3	2	3	2	1	1
35	48	1	4	1	1	3	5	5	4	3	2	3	2	3	2	1	1
36	74	2	4	1	1	3	0	5	3	3	2	3	2	3	2	1	1
37	71	2	4	3	1	3	0	5	3	3	2	3	2	3	2	1	1
38	80	2	5	3	1	3	5	2	2	3	2	3	2	3	3	1	1
40	1	2	2	3	2	2	0	5	2	2	2	1	3	2	1	1	1
41	8	2	2	3	2	3	0	5	2	2	2	3	3	3	1	1	1
42	2	2	2	3	2	3	0	5	2	2	2	3	3	2	1	1	1
43	42	2	2	4	2	3	0	6	2	2	2	2	3	3	1	1	1
44	44	2	2	4	2	1	0	3	2	2	2	3	3	3	1	1	1
45	43	2	2	5	2	3	0	3	2	2	2	3	3	3	1	1	1
46	13	2	2	3	2	2	0	5	2	1	2	3	3	4	1	2	1
47	39	2	2	3	2	3	0	5	2	1	2	3	3	3	1	1	1
48	4	2	2	3	2	3	0	5	2	1	2	3	3	2	1	1	1
49	5	2	2	3	2	3	4	5	1	2	2	3	3	2	1	1	1
50	29	2	4	4	2	3	3	5	2	2	2	3	3	3	3	1	1
51	30	2	4	4	2	3	3	5	2	2	2	3	3	3	1	1	1
52	9	2	1	3	2	3	0	5	2	2	2	3	3	3	1	1	1
53	10	2	0	3	2	3	0	5	2	2	2	3	4	3	1	1	1
54	35	1	2	4	2	3	0	5	3	2	2	3	4	4	1	1	1
55	23	2	2	3	2	3	0	5	4	2	2	3	4	3	1	1	1
56	24	2	2	2	2	3	2	5	4	2	2	3	4	3	1	1	1
57	25	2	2	3	2	3	0	2	4	2	2	3	4	3	1	1	1
58	45	2	2	5	2	2	0	1	4	2	2	3	4	3	1	1	1
59	11	2	2	3	2	3	0	5	2	2	2	3	4	3	1	1	1
60	36	2	2	3	2	3	0	5	2	2	2	3	4	4	1	1	1
61	15	2	2	4	2	3	3	5	2	2	2	3	4	3	1	1	1
62	16	2	2	4	2	3	4	5	2	2	2	3	4	3	1	1	1
63	37	2	2	3	2	3	5	5	2	2	2	3	4	4	1	1	1
64	26	2	2	3	2	3	0	4	2	2	2	3	4	3	1	1	1
65	28	2	2	3	2	3	1	2	2	2	2	3	4	3	1	1	1
67	7	2	2	2	2	3	4	5	2	1	2	3	4	2	1	1	1
68	12	2	3	3	2	3	4	5	2	2	2	3	4	3	1	1	1
69	14	2	2	3	2	3	0	5	2	1	2	3	4	3	1	1	1

Continued on following page

TABLE 1—*Continued*

ET[a]	DPN[a]	Relative mobility of[b]:														
		ADH	LGG	MPI	PGD	GLUD	NP1	NP2	ACP	IDH	PGI	FUM	G6P	PGM	GP1	IPO	ACO
70	22	2	0	4	2	3	4	5	2	2	2	3	3	3	2	1	1
71	38	2	2	4	2	3	4	5	2	2	2	3	4	4	1	1	1
72	18	2	2	4	2	3	0	5	2	3	2	3	3	3	1	1	1
73	41	2	2	4	2	2	0	5	2	3	2	3	3	3	1	1	1
74	19	2	2	4	2	3	0	5	2	2	2	3	3	3	1	1	1
75	27	2	2	3	2	3	0	3	2	1	2	3	4	3	1	1	1
76	3	2	2	3	2	3	2	5	2	2	2	3	3	2	1	1	1
77	6	2	4	4	2	3	2	5	2	2	2	3	3	2	1	1	1
78	20	2	2	3	2	3	4	5	2	3	2	3	3	3	1	1	1
79	21	2	2	3	1	3	4	5	2	2	2	3	3	3	1	1	1
80	33	2	5	4	2	3	4	5	2	3	2	3	3	3	3	1	1
81	34	2	5	4	2	3	4	4	2	2	2	3	3	3	3	1	1
82	31	2	2	4	2	3	6	5	2	2	2	3	3	3	3	1	1
83	32	2	2	4	2	3	6	5	2	2	2	3	4	3	3	1	1
84	40	2	2	4	2	2	0	5	2	2	2	3	3	3	3	1	1
85	72	2	3	3	1	3	5	5	4	2	2	2	2	3	2	1	1
86	70	2	4	3	1	3	5	5	3	3	2	2	2	3	2	1	1
87	17	2	2	4	1	3	0	5	2	2	2	3	3	3	1	1	1
88	82	1	5	1	1	3	0	5	4	3	2	2	2	4	2	1	1

[a] See Results for an explanation of ET and DPN designations.
[b] Mobilities are numbered in increasing order of anodal migration. See Materials and Methods for enzyme abbreviations.

comparison of each of these values with respect to serotype revealed a wide range of values. ETs within serotype 1/2a were the most diverse (h = 0.254) but still had only 61% of the diversity of the entire species. In contrast, the genetic diversity of all ETs representing strains of somatic antigenic type 1/2, regardless of flagellar antigen, was 0.395, or 94.2% of the total diversity. Therefore, much of the diversity of *L. monocytogenes* is due to differences between ETs representing strains with different flagellar antigens.

The genetic diversities of serotype 1/2b, 3b, and 4b ETs were 56.8, 39.1, and 49.6%, respectively, of the diversity of the entire collection of ETs. Likewise, serotype 1/2b, 3b, and 4b ETs had 93, 64.5, and 82%, respectively, of the diversity of serotype 1/2a ETs. The dendrogram (data not shown) generated from the matrix of distance coefficients revealed two distinct clusters of ETs, separated at a GD of 0.47, corresponding to ETGA and ETGB in Fig. 2. All strains of flagellar type a fell into ETGA, while ETGB contained all strains of flagellar type b. The one strain of flagellar antigen type c fell into ETGA (ET71; DPN38).

Many alleles of specific loci are associated exclusively, or nearly so, with specific flagellar antigens. For instance, by using G6P, a strain can be assigned to a particular flagellar antigen type with almost 100% accuracy. The one exception was the 1/2c isolate, which was indistinguishable from other isolates in ETGA on the basis of this one enzyme.

Isolates from food. Complete serotyping data were not obtained for the isolates from food; therefore, analyses by serotype were not done. Thirty-four ETs were identified among 56 isolates. When a dendrogram (not shown) was generated from the data, two distinct groups of *L. monocytogenes*, corresponding to ETGA and ETGB and separated by a GD of 0.403, were evident. The genetic diversities, mean numbers of isolates per ET, and average mismatches per ET are given in Table 4. Strains of some of the ETs were also present in the clinical isolates (Tables 2 and 3).

Epidemiologic significance. All seven clinical isolates from a listeriosis outbreak in Massachusetts in 1983 (11) were identical and were ET3. Clinical isolates from a 1985 outbreak in Los Angeles, Calif., which was associated with Mexican-style cheese were of a single ET (ET14), and *L. monocytogenes* of the same ET was isolated from the implicated cheese. These results agreed with phage typing results obtained during the outbreak (15). Only 5 (8%) of 61 surveillance isolates from Los Angeles collected during 1986 and 1987 were of this ET, demonstrating that the outbreak did not continue after removal of the implicated cheese from the shelves. The electrophoretic typing results documented the epidemiologic link between the cases and the contaminated product to which the affected persons were exposed, a link established by case control studies during the outbreak. Strains from the outbreaks in Switzerland (2) and Nova Scotia, Canada (21), were also ET14. In contrast, in 22 isolates obtained from patients during a listeriosis outbreak in Philadelphia, Pa., during 1987, we found 11 different ETs (23). This effectively ruled out a single common food as a cause of this outbreak.

We were also able to identify three clusters of three or more cases involving single ETs within a short time (1 month) and in a geographic area. Strains of two of these ETs (ET3 and ET17) were commonly isolated during the surveillance. Four strains of ET47 represented a cluster of cases caused by a strain not identified elsewhere during the surveillance. These data suggest that small, unrecognized clusters of disease from a single source are likely to exist (Gellin, submitted).

Independently of the Centers for Disease Control laboratory-based surveillance study, six cases of listeriosis were reported in which *L. monocytogenes* was isolated from patients and from foods to which the patients were exposed. In the comparison of the six isolates of *L. monocytogenes* from patients and products done at the Centers for Disease Control during 1986 and 1987 (Table 6), it was found that two patient-product pairs did not have matching ETs, indicating that the product was not the means of infection. For three pairs, the ETs of both isolates were the same but the pairs were of a common ET, making it less certain that infection was caused by the implicated product. The two isolates in one pair had a single unique ET (ET41).

TABLE 2. DPNs, ET designations, and serotypes of clinical and food product isolates of *L. monocytogenes* in ETGA

DPN	ET	Serotype	No. of strains			Source (reference)
			Surveillance[a]	Other clinical sources[b]	Foods[c]	
1	40	1/2a	0	1	0	Philadelphia outbreak (23)
2	42	1/2a	1	1	0	Philadelphia outbreak (23)
3	76	ND[d]	0	0	1	
4	48	1/2a	0	1	0	Philadelphia outbreak (23)
5	49	1/2a	1	0	1	
6	77	ND	0	0	1	
7	67	1/2a	15	3	0	
8	41	1/2a	4	0	0	
9	52	1/2a	0	1	1	
10	53	1/2a	1	0	0	
11	59	1/2a	1	0	0	
12	68	1/2a	4	0	0	
13	46	1/2a	1	0	0	
14	69	1/2a	1	1	0	Philadelphia outbreak (23)
15	61	1/2a	0	1	0	Perinatal case in rhesus monkey
16	62	1/2a	1	0	0	
17	89	ND	0	0	1	
18	72	ND	0	0	0	
19	74	ND	0	0	1	
20	78	ND	0	0	1	
21	79	ND	0	0	1	
22	70	3a	1	0	1	
23	55	1/2a	1	0	0	
24	56	1/2a	0	3	0	Perinatal cases in rhesus monkeys
25	57	1/2a	2	0	0	
26	64	1/2a	1	0	0	
27	75	ND	0	0	1	
28	65	1/2a	1	0	0	
29	50	1/2a	11	0	0	
30	51	1/2a	1	0	0	
31	82	ND	0	0	1	
32	83	ND	0	0	1	
33	80	ND	0	0	1	
34	81	ND	0	0	1	
35	54	1/2a	0	1	0	
36	60	1/2a	1	0	0	
37	63	1/2a	1	0	0	
38	71	1/2c	1	0	0	
39	47	1/2a	4	0	0	
40	84	ND	0	0	1	
41	73	ND	0	0	1	
42	43	1/2a	1	0	0	
43	45	1/2a	1	0	0	
44	44	1/2a	1	0	0	
45	58	1/2a	0	0	0	

[a] Isolates obtained during laboratory-based surveillance for listeriosis (1986 to 1987).
[b] Clinical isolates other than surveillance isolates received at the Centers for Disease Control during the surveillance period. Does not include isolates from known common-source outbreaks.
[c] Isolates from food that were obtained from the Food and Drug Administration and from foods for which an epidemiologic association with one or more patients with listeriosis was hypothesized. When multiple isolates with the same ET were obtained from the same source, only one representative strain was included.
[d] ND, Not determined.

TABLE 3. DPN, ET designations, and serotypes of clinical and food product isolates of *L. monocytogenes* in ETGB

DPN	ET	Serotype(s)	No. of strains			Source (reference)
			Surveillance[a]	Other clinical sources[b]	Foods[c]	
46	1	4b	18	8	0	
47	34	ND[d]	0	0	2	
48	35	ND	0	0	1	
49	3	4b	38	0	6	Massachusetts outbreak (11)
50	23	4b	2	1	0	
51	8	4b	2	0	0	
52	7	1/2b	14	2	3	Philadelphia outbreak (23)
53	27	1/2b	1	0	0	
54	31	ND	0	0	1	
55	20	4b	2	0	0	
56	25	4b	1	1	1	
57	2	1/2b	2	0	0	
58	22	1/2b, 3b	2	1	0	Philadelphia outbreak (23)
59	33	ND	0	0	1	
60	32	ND	0	0	1	
61	4	1/2b, 3b	26	5	2	
62	30	ND	0	0	1	
63	9	1/2b	0	1	0	
64	19	1/2b	1	0	0	
65	12	ND	0	1	0	
66	15	4b	3	5	1	Philadelphia outbreak (23)
67	13	4b	1	0	0	
68	17	1/2b, 3b	25	6	11	
69	18	3b	1	0	1	
70	86	ND	0	0	1	
71	37	ND	0	0	1	
72	85	ND	0	0	1	
73	14	4b	12	7	5	California, Philadelphia, Nova Scotia, and Swiss outbreaks (2, 15, 21, 23)
74	36	ND	0	0	1	
75	16	1/2b	8	0	1	
76	5	1/2b	1	0	0	
77	11	1/2b	2	0	0	
78	21	1/2b	1	0	0	
79	24	4b	1	0	0	
80	38	ND	0	0	3	
81	26	3b	0	1	0	Philadelphia outbreak (23)
82	88	ND	0	0	1	

[a] Isolates obtained during laboratory-based surveillance for listeriosis (1986 to 1987).
[b] Clinical isolates other than surveillance isolates received at the Centers for Disease Control during the surveillance period. Does not include isolates from known common-source outbreaks.
[c] Food isolates obtained from the Food and Drug administration and from foods for which an epidemiologic association with one or more patients with listeriosis was hypothesized. When multiple isolates with identical ETs were obtained from the same source, only one representative strain was included.
[d] ND, Not determined.

DISCUSSION

Analysis of the allelic variation of *L. monocytogenes* resulted in the generation of a dendrogram reflecting the genetic relationships among the strains examined. Grouping of ETs into recognizable clusters reflects nonrandom associations of alleles at different loci. For example, the association, in ETGA, of allele 2 or 3 at G6P and allele 2 at PGD and the association of allele 1 at G6P and allele 1 at PGD in ETGB reflect nonrandom association of the G6P and PGD alleles. Other, less obvious associations were detected by computer-assisted analysis of the data, resulting in a dendrogram reflecting these associations. Although this study was

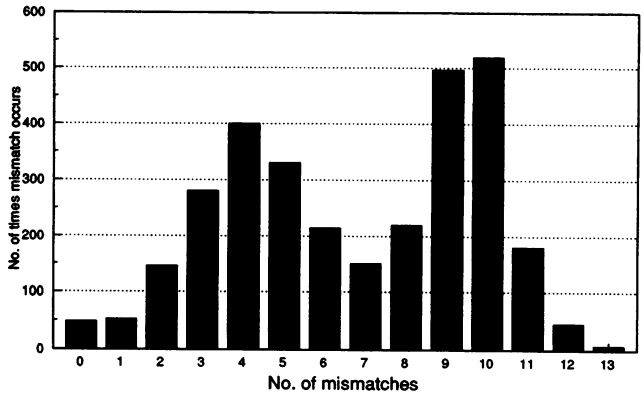

FIG. 1. Distribution of pairwise mismatches of alleles over all enzyme loci of *L. monocytogenes*.

limited to strains isolated primarily in the United States over a short time span (5 years), the isolation of strains of a single ET over this period and from varied geographic areas is indicative of the stability of individual clones during the period studied.

In multilocus enzyme studies of *Escherichia coli* and *Legionella pneumophila*, little correlation was observed between the serologic profiles and ETs (9, 14, 26). In contrast, a high correlation between these characteristics was observed in *L. monocytogenes*. Except in two instances, no ET contained strains from more than one serotype. No strains of serotype 1/2b, 3b, or 4b were in ETGA, and no strains of serotype 1/2a were in ETGB, indicating that the flagellar antigens are at least as stable in composition as the enzymes that were assayed for in this study. It is logical to conclude that these serological properties of *L. monocytogenes* are highly conserved phenotypic characteristics and not simply character states, as suggested by Selander et al. for *E. coli* and *L. pneumophila* (26). The somatic antigens do not exhibit such a high degree of correlation with ET clusters. Strains of a single antigenic group based solely on somatic antigens were distributed over many ETs, and ETs representing strains of differing serotypes were often closely related to each other.

TABLE 4. Genetic diversity, mean number of alleles per locus, and mean number of strains per ET for clinical and product isolates of *L. monocytogenes* in ETGA, ETGB, and all ETs

Genetic group	Genetic diversity	Mean no. of alleles/locus	No. of strains/ET
ETGA			
Clinical isolates[a]	0.264	3.13	2.12
Isolates from food[b]	0.249	1.94	1.00
All isolates[c]	0.275	3.31	1.80
ETGB			
Clinical isolates	0.259	2.31	8.45
Isolates from food	0.256	1.94	2.19
All isolates	0.266	2.50	6.73
All ETs			
Clinical isolates	0.428	3.75	5.20
Isolates from food	0.410	2.69	1.67
All isolates	0.428	3.75	4.11

[a] Analyses of ETs representative of all clinical strains examined, except strains from common-source outbreaks.
[b] Analyses of ETs representative of all isolates from food products examined.
[c] Analyses of ETs representative of the combination of all clinical and all food product isolates of *L. monocytogenes*.

The benefits of using electrophoretic typing in epidemiologic studies are evident. Unlike with phage typing, all strains of *L. monocytogenes* are typeable by this method. We were also able to document the background distribution of ET phenotypes in the U.S. population by examining a large number of isolates collected during a specified period in diverse geographic locations. The implication of the finding that ETs of isolates from patients and isolates from a presumed source are identical varies depending on whether

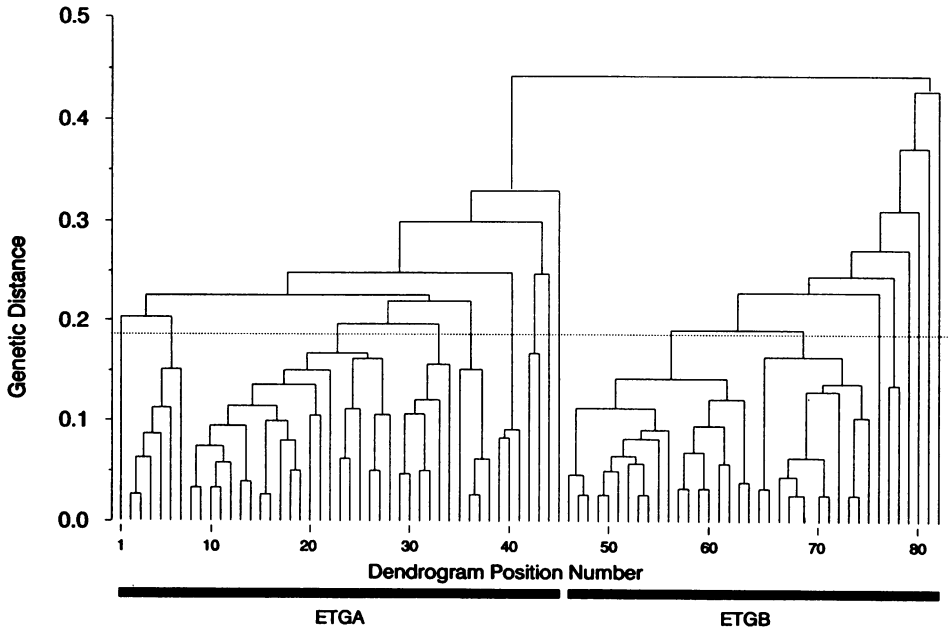

FIG. 2. Dendrogram showing genetic relationships among ETs of *L. monocytogenes*.

TABLE 5. Genetic diversity, number of alleles per locus, and mean number of strains per ET for all serotype combinations of clinical isolates of *L. monocytogenes*

Serotype(s)	Genetic diversity	No. of alleles/locus	No. of ETs	No. of strains/ET
1/2a, 1/2b, 1/2c, 3a, 3b, 4b	0.428	3.750	55	5.2
1/2a, 1/2b, 1/2c	0.395	3.625	42	3.8
1/2b, 3b, 4b	0.244	2.250	23	8.0
1/2a, 3a	0.258	3.125	30	2.3
1/2a	0.254	3.063	29	2.4
1/2b	0.238	1.875	12	8.5
3b	0.164	1.438	5	1.4
4b	0.208	1.813	10	10.0

the ET is rare or prevalent in the population. Recently, as part of another population-based surveillance study, a case of listeriosis was identified and turkey franks were implicated as the means of infection (10). The ETs of an isolate from a patient and isolates from an opened package of turkey franks in the patient's refrigerator were identical, and this type had not been observed during 1986 and 1987. Subsequently, strains of this ET were isolated from unopened packages of this product. *L. monocytogenes* of the same ET was isolated from several different foods in the patient's refrigerator, suggesting that cross-contamination of these products had occurred and that isolation of *L. monocytogenes* from opened packages of food is not sufficient to identify the source of infection in any single case.

Piffaretti and co-workers, in a similar study of primarily European isolates, inferred from their data that differences in virulence may exist within ETGB (19). This supposition was based heavily on the predominance of ET3 and ET14 strains (their ET7 and ET1, respectively) within their data base and the close relationship that they found between these ETs. The claim was made that strains of these two ETs accounted for two-thirds of the invasive disease in Europe. However, those researchers failed to emphasize that the number of strains within ET14 was accounted for by the large number of known common-source epidemic strains studied (62 of 89). Furthermore, the method of collection and the time span over which the other isolates were obtained were not detailed, making such a conclusion largely unsupported. In our analysis, the two ETs were not as closely related (a GD of approximately 0.1 versus a GD of 0.2).

Since the other investigators did not report the relative mobilities of the enzymes that defined their ETs, it is difficult to reconcile these differences. The discrepancies in results could be due to a difference in the overall method of strain collection, use of different enzymes in the two studies, or a difference in the experimental conditions (such as different buffer systems). In England, the predominant serotype causing disease is 4b (17), while in the United States a relatively even distribution among serotypes 1/2a, 1/2b, and 4b has been observed, reflecting geographic differences in the distribution of serotypes and, therefore, ETs.

TABLE 6. ETs of isolates from patients and from food products with a possible epidemiologic link to the patients

Case no.	ET of patient-derived stain (no. of surveillance isolates)[a]	ET of product-derived strain
1	1 (18)	3
2	15 (3)	14
3	14 (12)	14
4	14 (12)	14
5	3 (38)	3
6	41 (0)	41

[a] Number of surveillance isolates with the same ET as the isolate from the patient.

The enzymes that were common to the two studies were approximately equivalent with respect to genetic diversity at the respective loci (4). However, two of the three loci (LGG and NP1) at which we found differences in electrophoretic mobility between these two ETs were not assayed for by Piffaretti et al. These variations could account for the differences in clustering and the larger GD we found between these two clones. Also, comparison of the electrophoretic profiles of our ET3 and ET7 revealed that these two ETs differ only at LGG and NP1. Piffaretti et al. listed their ET7 (our ET3) as being composed of both serotype 4b and 1/2b strains. Since our ET3 is composed of serotype 4b strains and our ET7 is composed of serotype 1/2b strains, we suggest that their ET7 is made up of these two different ETs. This would also vitiate the suggestion that two ETs cause two-thirds of the invasive disease in Europe. We did find that strains of ET3 and ET14 were common in our population-based study. Six other ETs, however, were as prevalent as ET14. These six ETs are distributed among a number of clusters of ETs and span the dendrogram.

In an analysis of a subset of the strains we studied, all ETs with more than 10 strains were examined and no trends in ET distribution between geographic areas, perinatal versus nonperinatal cases, early-onset versus late-onset neonatal cases, type of clinical presentation, or fatal versus nonfatal infections were identified (Gellin, submitted). Thus, the supposition that the cluster of ETs which includes ET14 and ET3 is a virulent family of clones is not supported by our work and brings into question the biologic significance of subtle differences in GDs and clustering.

It is, however, interesting that three of five outbreaks of listeriosis were caused by a single clone of the organism and the possibility that ET14 represents a particularly virulent clone cannot be ruled out. Approximately 8% of the food isolates were also ET14. Also, clinical isolates had an average of 5.2 strains per ET, compared with 1.67 strains per ET for food isolates ($P = 0.04$). This difference was also seen within ETGA and ETGB.

The similar genetic diversities revealed by the data obtained from isolates from food and patients in our study suggest that both groups are representative of the species as a whole. This could indicate that clinical virulence is restricted to subpopulations of *L. monocytogenes*, or it may reflect the scope of distribution and consumption of individual food products. *L. monocytogenes* is widespread in the environment, and our sample does not encompass many niches where the organism thrives. The food isolates we analyzed may not reflect a cross-section of the organism that contaminates our food supply. For instance, we examined only one isolate from meat products, which have been implicated as contributing to sporadic cases of listeriosis caused by ingestion of contaminated foods (10, 22).

The species is divided into two distinct genetic groups at a GD of 0.440, a result which is confirmed by the work of Piffaretti et al. (19). No direct biologic significance, such as different virulence factors, can be attributed to the genetic structure, either at this level or at a lower GD. The dendrogram presented here was generated by weighting the data such that less-variable loci were given more weight in determining GDs. When unweighted data were analyzed, single ETs were sometimes shifted in genetic relatedness to

other ETs or clusters of ETs; however, the division between ETGA and ETGB not only remained intact but increased from a GD of 0.440 to 0.612 (data not shown).

Other researchers have attempted to relate differences in the disease syndromes of listeriosis to differences in serotype. Albritton et al. (1) found that two distinct syndromes were associated with perinatal listeriosis. Early-onset (mean age at onset, 1.3 days) and late-onset (mean age at onset, 14.0 days) listeriosis cases revealed significant differences with respect to mean birth weight, mortality, isolation of listerias from the mother, and obstetric complications. Strains isolated from early- and late-onset cases appeared to differ with respect to serotype. Specifically, serotype 1/2a (corresponding to ETGA) strains were infrequently associated with late-onset perinatal listeriosis. Because very few late-onset neonatal cases were identified in the set of data described here, we could make no such comparison. Evidence derived from data obtained during the laboratory-based surveillance study, however, suggests that a greater proportion of perinatal cases results from strains of ETGB (odds ratio, 2.3; $P = 0.03$) (Gellin, submitted). The pathophysiologic basis for this potential difference is not understood. No other clinical or epidemiologic differences (syndrome, case-fatality ratio, or geographic clustering) were detected when the cases caused by strains from ETGA and ETGB were compared.

Furthermore, a monoclonal antibody made against the hemolysin of a serotype 1/2a strain reacted only with strains of this serotype and a strain of serotype 1/2c (B. Swaminathan, personal communication). These data indicate that there may be differences in pathogenesis between the two groups. If we assume that the loci that we examined are neutral, that is, there is no selective advantage of one electrophoretic variant over another, then the clonality of *L. monocytogenes* is possibly due to intense selections at other loci. Furthermore, we must consider the possibility that these other loci are related to virulence or to growth characteristics. Since we have shown that *L. monocytogenes* is composed of two distinct genetic groups, it seems prudent to suggest that future studies done with the organism be designed to include representatives of both electrophoretic groups of organisms.

Whether the distribution of ETs that we observed will change remains to be seen. The prevalence of certain ETs in the populations we studied may be due to several factors. It may be attributed to widespread distribution of these ETs in the environment or wide distribution of single or multiple food items contaminated with the particular ET, or it may indicate variability in virulence between strains of different ETs. Further study of the distribution of ETs over a greater temporal and geographic span and from a larger selection of patients and food items may answer these questions and will enhance our knowledge of the distribution of *L. monocytogenes* in the environment, in the food supply, and in human illness. To address these questions and others, another large-scale surveillance project involving examination of foods from the refrigerators of patients with listeriosis and determination of the serotypes and ETs of any *L. monocytogenes* isolates obtained is being conducted.

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